UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: May 8, 2012

SUBJECT: Sulfuryl fluoride, Immunotoxicity study in mice

PC Code: 078003
Decision No.: 459187
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0056214
MRID No.: 48646001

DP Barcode: 397237
Registration No.: N/A
Regulatory Action: N/A
Submission No.: N/A
CAS No.: 2699-78-8
40 CFR: N/A

FROM: **Yung G. Yang, Ph.D.**
Risk Assessment Branch VI
Health Effects Division (7509 P)

THROUGH: **Felecia Fort, Chief**
Risk Assessment Branch VI
Health Effects Division (7509 P)

TO: **Christina Swartz, Chief**
Risk Assessment Branch II
Health Effects Division (7509P)
and
Dana Friedman
RMIB2
Pesticide Re-Evaluation Division (7508P)

I. CONCLUSIONS

The immunotoxicity study in mice for sulfuryl fluoride (MRID 48646001) has been reviewed. It is classified as acceptable/guideline and satisfies guideline requirements for an immunotoxicity study (OPPTS 870.7800).

II. BACKGROUND and ACTION REQUESTED

An immunotoxicity study on sulfuryl fluoride (MRID 48646001) was submitted. RAB VI was asked to review and prepare a DER for this study.

III. RESULTS AND DISCUSSION

The immunotoxicity study in mice for sulfuryl fluoride (MRID 48646001) has been reviewed. The DER is attached and an executive summary is as follows:

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID 48646001), sulfuryl fluoride (99.7% a.i., Lot ZE27160101) was administered to female Crl:CD1 (ICR) mice via inhalation at exposure levels of 0 (control), 10, 30, or 100 ppm (the actual doses received were 0, 10.3, 30.3, 100.5 ppm, respectively) for six hours/day over five days/week, for 28 days (20 exposures). Four days before necropsy, animals in all groups were immunized with a suspension of sheep red blood cells (SRBC) by intravenous injection ($5x10^8$ SRBC/animal, 0.2 mL/animal). Animals in the positive control group received cyclophosphamide by intraperitoneal injections (ip) at doses of 20 mg/kg/day on Days 24-28. All animals were evaluated for mortality, clinical signs, body weight changes, food consumption, hematology, and gross pathology. On Day 29, blood samples from all animals were collected from the retro-orbital sinus at the scheduled necropsy. Immunotoxicity was assessed using an Enzyme-Linked Immunosorbent Assay (ELISA) that measured the concentration of serum anti-SRBC immunoglobulin M (IgM). The organ weights of the spleen, thymus, and brain were determined at necropsy.

No treatment-related effects were noted at the 10, 30, or 100 ppm test groups. Although no signs of toxicity were seen, the concentration of 100 ppm was considered adequate based on previous inhalation toxicities studies in mice.

The systemic toxicity NOAEL is 100 ppm, the highest dose tested; the LOAEL could not be determined (>100 ppm).

For immunotoxicity, there were no treatment-related effects on serum anti-SRBC IgM antibody levels as measured by an ELISA, or thymus and spleen weights. There was a high inter-individual variability noted in all the treatment groups as well as in the control group. Evaluation of individual animal data of this study did not show any trend or distribution that would demonstrate a significant suppression of anti-SRBC IgM response. The positive control group confirmed the ability of the test system to detect immuno-suppressive effects and confirmed the validity of the study design.

The Natural Killer (NK) cells activity was not evaluated in this study. The toxicology database for sulfuryl fluoride does not reveal any evidence of treatment-related effects on the immune system. The overall weight of evidence suggests that this chemical does not directly target the immune system. Under HED guidance, a NK cells activity assay is not required at this time.

Under conditions of this study, the immunotoxicity NOAEL is 100 ppm, the highest dose tested; the LOAEL was not established (>100 ppm).

This immunotoxicity study is classified as **acceptable/guideline** and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in the mouse.

DATA EVALUATION RECORD
Sulfuryl fluoride
PC Code: 078003
TXR#: 0056214
MRID#: 48646001

Study Type: Immunotoxicity - Mouse
OPPTS 870.7800

Prepared for

Health Effects Division
Office of Pesticide Programs
U. S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Tetrahedron Incorporated
1414 Key Highway, Suite B
Baltimore, MD 21230

Principal Reviewer: Nasrin Begum Date: 2/8/2012
Nasrin Begum, Ph.D.

Secondary Reviewer: Date: 2/12/2012
William Burnam, M. S.

Quality Assurance: Date: 2/15/2012
Reneé Bouwkamp, M.S., PE

Contract Number: EP-W-10013
Work Assignment No. : WA-0-01

Task Number: 1-1-83
EPA Reviewer//WAM: Swartz/Scollon//Brunsman/Farwell

Disclaimer

This review may have been altered by the EPA subsequent to the contractors' signature above.

4

EPA Reviewer: Yung G. Yang, Ph. D. **Signature:** Y G Y
Risk Assessment Branch VI, Health Effects Division (7509P) **Date:** 5/3/12
EPA Work Assignment Manager: Lori Brunsman **Signature:** Lori Brunsman
Science Info. Management Branch, Health Effects Division (7509P) **Date:** 5/3/12

Template version 09/11

TXR #: 056214

DATA EVALUATION RECORD

STUDY TYPE: Immunotoxicity [inhalation] - Mice; OPPTS 870. 7800

PC CODE: 078003 **DP BARCODE:** D397237

TEST MATERIAL (PURITY): Sulfuryl fluoride (99.7% wt/wt., Lot ZE27160101))

SYNONYMS: N,N -Bis(2-chloroethyl)tetrahydro-2H -1,3 ,2-oxazaphosphorin-2-amine-2-oxide hydrate (1; 1), Cyclophosphamide hydrate

CITATION: Boverhof D.R., Murray J.A., Bell M. P., Hukkanen R., Kriger S.M., Sura R. (2011) Assessment of Immunotoxic Potential Using the Sheep Red Blood Cell Assay after 28-Day Inhalation Exposure Using Female Crl:CD1(ICR) Mice. Toxicology & Environmental Research and Consulting, The Dow Chemical Company Midland, Michigan 48674. Study No. 111115, October 24, 2011. MRID 48646001. Unpublished.

SPONSOR: Dow AgroSciences LLC, Indianapolis, Indiana

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID 48646001), sulfuryl fluoride (99.7% a.i., Lot ZE27160101) was administered to female Crl:CD1 (ICR) mice via inhalation at exposure levels of 0 (control), 10, 30, or 100 ppm (the actual doses received were 0, 10.3, 30.3, 100.5 ppm, respectively) for six hours/day over five days/week, for 28 days (20 exposures). Four days before necropsy, animals in all groups were immunized with a suspension of sheep red blood cells (SRBC) by intravenous injection ($5x10^8$ SRBC/animal, 0.2 mL/animal). Animals in the positive control group received cyclophosphamide by intraperitoneal injections (ip) at doses of 20 mg/kg/day on Days 24-28. All animals were evaluated for mortality, clinical signs, body weight changes, food consumption, hematology, and gross pathology. On Day 29, blood samples from all animals were collected from the retro-orbital sinus at the scheduled necropsy. Immunotoxicity was assessed using an Enzyme-Linked Immunosorbent Assay (ELISA) that measured the concentration of serum anti-SRBC immunoglobulin M (IgM). The organ weights of the spleen, thymus, and brain were determined at necropsy.

No treatment-related effects were noted at the 10, 30, or 100 ppm test groups. Although no signs of toxicity were seen, the concentration of 100 ppm was considered adequate based on previous inhalation toxicities studies in mice.

The systemic toxicity NOAEL is 100 ppm, the highest dose tested; the LOAEL could not be determined (>100 ppm).

For immunotoxicity, there were no treatment-related effects on serum anti-SRBC IgM antibody levels as measured by an ELISA, or thymus and spleen weights. There was a high inter-

individual variability noted in all the treatment groups as well as in the control group. Evaluation of individual animal data of this study did not show any trend or distribution that would demonstrate a significant suppression of anti-SRBC IgM response. The positive control group confirmed the ability of the test system to detect immuno-suppressive effects and confirmed the validity of the study design.

The Natural Killer (NK) cells activity was not evaluated in this study. The toxicology database for sulfuryl fluoride does not reveal any evidence of treatment-related effects on the immune system. The overall weight of evidence suggests that this chemical does not directly target the immune system. Under HED guidance, a NK cells activity assay is not required at this time.

Under conditions of this study, the immunotoxicity NOAEL is 100 ppm, the highest dose tested; the LOAEL was not established (>100 ppm).

This immunotoxicity study is classified as **acceptable/guideline** and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in the mouse.

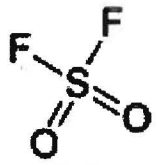
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Sulfuryl fluoride

Description:	Gas, no description provided
Lot #:	ZE27160101
Purity:	99.7% wt/wt
Compound Stability:	35 days at room temp., demonstrated in a previous study
CAS # of TGAI:	2699-78-8
Structure:	



2. Vehicle and/or positive control: Vehicle is air. Positive control is cyclophosphamide monohydrate (CP, Sigma-Aldrich, St. Louis MO, Batch# 079K1569).

3 Test animals:

Species:	Mouse, female
Strain:	Crl:CD1 (ICR)
Age/weight at study initiation:	Approx. 7 weeks, 15. 8-18. 6 grams at initiation of dosing
Source:	Charles River Laboratories (Portage, Michigan).
Housing:	Housed individually in suspended, stainless steel, wire-mesh cages.
Diet:	Certified rodent powdered and irradiated diet Diet #5002 (PMI Nutrition International,St. Louis, Missouri), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 22°C±3° **Humidity:** 40-70% **Air changes:** 10-15/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	13 days

B. STUDY DESIGN:

1. In life dates - Start: July 18, 2011 (initiation of test substance administration, Day 0)
End: August 15, 2011 (scheduled necropsy, Day 28)

2. Animal assignment: Animals were assigned to the test groups noted in Table 1. All available animals were weighed and examined for physical abnormalities. Suitable animals were selected for assignment using a computerized randomization procedure based on body weight stratification in a block design.

TABLE 1: Study design [a]

Group	Target concentration (ppm)	Actual dose to animal (ppm)	# Female
1	0 (control)	0	10
2	10	10.3	10
3	30	30.3	10
4	100	100.5	10
5	Positive control[b]	20 mg/kg/day[b]	10

[a] Data obtained from page 16 of the study report.

[b] Positive control animals were administered cyclophosphamide monohydrate in sterilized saline by intraperitoneal injections at doses of 20 mg/kg/day on Days 24-28.

7

3. **Dose selection:** The report stated that "The high exposure level of 100 ppm was selected to produce some measureable sign of general toxicity on body weight and brain histopathology without producing significant stress, malnutrition, or fatalities. Exposure levels above 100 ppm were considered to exceed the maximum tolerated concentration for sulfuryl fluoride as mortality was observed in a previously conducted 2 week study at an exposure level of 300 ppm. The remaining exposure levels were expected to provide dose-dependent information on any treatment-related immunotoxic effects that may be observed in the high-exposure group. These exposure levels (10, 30, and 100 ppm) were also consistent with exposure levels from a previous 90-day inhalation study in mice. The low exposure concentration was expected to be a no-observed-effect level (NOEL)."

4. **Test material administration:** The test substance was delivered using mass flow controllers at a set rate to exposure chambers and was mixed with air to achieve proper dosage. The mice were exposed to the aerosolized test material in $2m^3$ stainless steel and glass Rochester-type whole-body inhalation exposure chambers under dynamic airflow conditions for 6 hours per day, 5 days per week for 4 consecutive weeks (for a total of 20 exposures). Prior to exposure, the chamber was checked to ensure that a uniform distribution of SO_2F_2 was present throughout the breathing zone of the animals.

5. **Generation of the test atmosphere / chamber description:** The test substance was dissolved in air (the vehicle) and was metered (calibrated Agilent Gas Chromatograph 6890A) into a nozzle and was maintained at approximately 450 liters per minute which was sufficient to provide the normal concentration of oxygen to the animals. A differential pressure transducer was calibrated with a gas meter (Singer Aluminum Diaphragm Meter, Model AL-2300) prior to the start of the study. Chamber temperature and relative humidity was measured with a resistance temperature device (RTD) and a humidity sensor (Omega HX94C, Omega Engineering Inc), respectively, approximately once each hour. All air flows are monitored continuously by means of computer-controlled mass-flow meters.

   Test atmosphere concentrations – The test atmosphere provide a sufficient number of air changes per hour [minimum air flow was 417 liters/min, maximum was 481 liter/min]. Under such test conditions steady state is attained within approximately one minute of exposure ($t_{99\%}$ = 4.6 x chamber volume/flow rate; McFarland, 1976). Based on the 450 liter per minute flow rate, the theoretical equilibrium time to 99% (T_{99}) of the target concentration was 20.5 minutes.

   Analysis of the test atmosphere – The concentration of the test substance was determined by a calibrated Agilent Gas Chromatograph 6890A twice per hour. The samples from the chamber were taken from the vicinity of the breathing zone using an air flow rate of 450 liter/min.

   Particle size determination – The test substance is gas.

   Chamber Concentration – The analytically mean determined chamber concentration values for the 0, 10, 30, and 100 ppm exposure chambers were 0, 10.3 ± 0.5,30.3 ± 1.0, and 100.5 ± 4.6 ppm sulfuryl fluoride, respectively (mean ± standard deviation). No sulfuryl fluoride was detected in the control (0 ppm) chamber samples at a concentration exceeding the lower limit of quantitation (LLQ) of 0.25 ppm. The nominal concentrations were 10.4, 32.5, and 108.6 ppm for the 10.3, 30.3, and 100.5 ppm exposure chambers, respectively

8

6. **Statistics:** "Body weights, food consumption, organ weights, hematologic data (red blood cell indices and differential white blood cell counts excluded) and the SRBC ELISA data were evaluated by Bartlett's test (α =0.01) for equality of variances. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric (Steel and Tonie, 1960) or nonparametric (Hollander and Wolfe, 1973) analysis of variance (ANOVA). If significant at alpha = 0.05, the ANOVA was followed respectively by Dunnett's test (alpha = 0.05; Winer, 1971) or the Wilcoxon Rank-Sum test (alpha = 0.05; Hollander and Wolfe, 1973) with a Bonferroni correction (Miller, 1966) for multiple comparisons to the control. The experiment-wise alpha level as reported for these two tests. For the positive control data was compared to the negative control data in an analysis that was separate from the treated groups and negative control. DCO incidence data (scored observations only) was statistically analyzed by a z-test of proportions comparing each treated group to the control group (alpha = 0.05; Bruning and Kintz, 1987). Descriptive statistics only (means and standard deviations) were reported for chamber concentration, temperature, relative humidity, and airflow, body weight gains, RBC indices, and differential WBC counts. Statistical outliers were identified by a sequential test (alpha = 0.02; Grubbs, 1969), but routinely excluded only from feed consumption statistics. Outliers may have been excluded from other analyses only for documented, scientifically sound reasons." From page 26 of the study.

   The Reviewer considers these analyses to be appropriate.

C. METHODS:

1. **Observations:** Animals were inspected at least once daily, once on weekends and holidays, for mortality and moribundity, and at least once daily for clinical signs for animals exposed to the test substance. Detailed physical examinations were performed at least weekly.

2. **Body weight:** Animals were weighed twice weekly, beginning before the study, and ending just prior to the scheduled necropsy. Weight gains were calculated relative to Day 1. Positive control animals were weighed once a week and at necropsy.

3. **Food/water consumption:** Food consumption for each animal (except the group exposed to cyclophosphamide) was determined weekly (twice in the first week). Mean daily diet consumption was calculated as:

   Feed consumption (g/day) = (initial weight of feed container - final weight of feed container) / (# of days in measurement cycle) (# of animals per cage)

   Water consumption was not measured.

4. **Sacrifice and pathology**: Animals were anesthetized on Day 29 by a mixture of isoflurane and medial oxygen followed by decapitation. Blood samples from all animals were collected from the retro-orbital sinus at the scheduled necropsy at the time of euthanasia for possible IgM antibody analysis using Enzyme-Linked Immunosorbent Assay (ELISA). The blood was processed to serum, and frozen at $\leq$ -74°C until analyzed.

   a. Gross necropsy: A limited necropsy was performed. It included examinations of external tissue and all orifices, the eyes *in situ*, and an examination of the thoracic and abdominal

cavities. All visceral tissues were dissected from the carcass, re-examined and selected tissues were incised. Brain, spleen, and thymus weights were measured.

b. Tissue preparation/histopathology: Representative samples of the brain, thyroid, spleen, thymus, sternum, mesenteric lymph node, and Peyer's patch ((gut-associated lymphoid tissue (GALT)) were collected and preserved in neutral, phosphate-buffered 10% formalin.

A histophath examination was not performed.

5. **Hematology:** At the scheduled necropsy, blood samples were collected. Samples were mixed with ethylenediaminetetra acetic acid and prepared with a Wright-Giemsa stain. The following hematological parameters were determined in peripheral blood:

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet (thrombocyte) count	X	Reticulocyte count
X	Blood clotting measurements		
X	(Thromboplastin time) (Hepato-Quick)		
	(Clotting time)		
	(Prothrombin time)		

6. **Immunotoxicity:** All animals were immunized on Day 24 with SRBC by injection into the lateral tail vein ($5x10^8$SRBC/animal, 0.2 mL/animal dose volume), animals were not fasted. On Day 29, blood samples from all animals were collected from the retro-orbital sinus at the scheduled necropsy. Immunotoxicity was assessed using an Enzyme-Linked Immunosorbent Assay (ELISA) that measured the concentration of serum anti-SRBC IgM.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment related clinical signs were noted in any of the control or test groups.

2. **Mortality:** No mortalities or unscheduled deaths were noted.

B. Body weight and weight gain: No effect on mean body weight or mean bodyweight gain parameters was observed in the test groups when compared to the control.

One individual in the control group (#7248) was considered a statistical outlier during measurements conducted on day 19 of the study. The result was included in the statistical calculations for the group.

C. FOOD/WATER CONSUMPTION:

1. **Food consumption:** No effect on food consumption was observed in the test groups when compared to the control.

2. **Water consumption:** Water consumption was not measured.

D. **GROSS NECROPSY:** There were no treatment-related gross pathologic observations. All gross pathologic observations were considered to be spontaneous alterations, unassociated with exposure to sulfuryl fluroide.

1. **Organ weight:** There was no relevant change in mean terminal body weight when compared to controls. Organ weights of treated animals were comparable to the ones of the controls.

   One animal in the 100 ppm group (#7277) that spleen weight was considered a statistical outlier. The result was included in the statistical calculations for the group.

   Subjects in the positive control displayed decreases in absolute (30.8%) and relative (28.9%) spleen weights and decreases in absolute (39.6%) and relative (39.5%) thymus weights when compared to control animals when compared to control animals.

E. **BLOOD TEST:**

1. **Hematology:** There were no treatment-related changes in any of the hematologic parameters.

   One individual in the 10 ppm group (#7258) had a reticulocyte count that was considered a statistical outlier. The result was included in the statistical calculations for the group.

2. **Anti-SRBC Antibody Response:** There were no statistically significant changes noted in anti-SRBC IgM concentrations in female mice at any concentration. A single animal in the control group (#7244) had a high concentration of anti-SRBC IgM that was identified as a statistical outlier; however, removal of this data point did not alter the analysis results or interpretation.

Table 2. Results of the ELISA in mice administered sulfuryl fluroide via inhalation for 28 days [a] Mean anti-SRBC IgM level (units/mL±SD)

Stat	0 ppm	10 ppm	30 ppm	100 ppm	20 mg/kg CP
Mean±SD	8535±6318	6757±2772	5778±2375	7999±4377	476*±248 (94.4%↓)
SE	1998	876	751	1384	78
N	10	10	10	10	10

[a]Data obtained from pages 53-54 of the study report.
* Statistically different ($p \leq 0.05$) from the control.

The group mean IgM result from mice dosed with the positive control, cyclophosphamide monohydrate, was greater than 90% lower than the vehicle control group, showing it was valid for evaluating immuno-supression.

Natural Killer (NK) cells activity assay: Not performed.

11

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS: The study authors concluded that no impairment of the immunological IgM response was observed after immunization with SRBC of animals treated with Sulfuryl fluoride via inhalation at dose levels up to 100 ppm for at least 28 days (corresponding to approximately 100.5 ppm). Therefore, Sulfuryl fluoride was considered not to have an immunotoxic potential.

B. REVIEWER COMMENTS: The systemic toxicity LOAEL of sulfuryl fluoride could not be determined. All of the dosage levels results were similar to the negative control. Although no signs of toxicity were seen, the concentration of 100 ppm was considered adequate based on previous inhalation toxicities studies in mice.

The systemic toxicity NOAEL is 100 ppm, the highest dose tested; the LOAEL could not be determined (>100 ppm).

For immunotoxicity, there were no treatment-related effects on serum anti-SRBC IgM antibody levels as measured by an ELISA, or brain, thymus, and spleen weights. There was a high inter-individual variability noted in all the treatment groups as well as in the control group. Evaluation of individual animal data of this study did not show any trend or distribution that would demonstrate significant suppression of anti-SRBC IgM response. The positive control group confirmed the ability of the test system to detect immuno-suppressive effects and confirmed the validity of the study design.

The Natural Killer (NK) cells activity was not evaluated in this study. The toxicology database for Sulfuryl fluoride does not reveal any evidence of treatment-related effects on the immune system. The overall weight of evidence suggests that this chemical does not directly target the immune system. Under HED guidance, a NK cells activity assay is not required at this time.

Under conditions of this study, the immunotoxicity NOAEL is 100 ppm, the highest dose tested; the LOAEL was not established (>100 ppm).

C. STUDY DEFICIENCIES: No deviations were noted.